pleated conformation in amyloidogenic polypeptides" (Wisniewski and Frangione, 1992). It would be useful to be able to replace or augment the activity of the chaperones where necessary and to counteract the activity of pathological chaperones when present.

C1 cd

Please rewrite the paragraph at column 2, lines 51-63, as follows:

Recent reports suggest that monoclonal antibodies (mAb) can have chaperone-like activity. The feasibility of using monoclonal antibodies to assist in the in vitro refolding process of guanidine-denatured S-protein was reported recently (Carlson and Yarmush, 1992). Previously, Blond and Goldberg (1987) used monoclonal antibodies as a tool in the identification and characterization of folding steps that involve the appearance of local native-like structures in $B_2$ subunit of [tryptophansynthase] tryptophan-synthase. Since the mAb is epitope specific, the use of mAb provides more specificity than molecular chaperones. mAbs can be sought and engineered (Haber, 1992) that bind to the particular epitope in the protein of interest that is involved in the folding process.

C2

Please rewrite the paragraph from column 2, line 64, to column 3, line 7, as follows:

The main difference between mAbs and molecular chaperones is that the latter [does] do not bind to native proteins and [is] are capable of interacting with many different polypeptide chains without exhibiting an apparent sequence preference (Goloubinof et al., 1989). Moreover, chaperones suppress aggregation but do not redissolve aggregate already present. Similar behavior was recently reported for α-crystalin which, similar to other chaperones, does not react with active proteins, but forms a stable complex with denaturing or partially unfolded proteins, stabilizing against further aggregation (Rao et al., 1994).

C3

Please rewrite the paragraph at column 3, lines 42-48, as follows:

According to the present invention, a method is provided of selecting an anti-aggregation molecule such as a monoclonal antibody, a genetically engineered antibody fragment or a peptide which mimics the binding site of an antibody. These anti-aggregation molecules are able to bind to a native target molecule epitope with a high binding constant and must be non-inhibitory to biological activity of the target molecule.

C4

Please rewrite the paragraph at column 4, lines 1-7, as follows:

FIG. 2 is a bar graph of the time course of denaturation of Carboxypeptidase A after exposure at 50° C.; the residual esterase (single cross-hatch bars) and peptidase (open bars) enzymic activity of CPA was measured at two intervals of incubation at 50° C.; the amount of residual soluble enzyme was determined by sandwich [ELIAS] ELISA (bars of diagonal lines);

Please rewrite the paragraph at column 4, lines 15-21, as follows:

FIG. 4 is a bar graph of the effect of epitope location on the maintenance of the enzymic activity of heat-exposed Carboxypeptidase A; increasing amounts of monoclonal antibodies $CP_{10}$ [(single cross-hatch bars)] (bars with diagonal lines) and $CP_9$ [(diagonal lines)] (open bar) and unrelated IgG [(bars with diagonal lines)] (cross-hatched bars) were added to CPA before exposure to 50° C. for one hour and esterase enzymic activity was measured;

Please rewrite the paragraph at column 4, lines 22-33, as follows:

FIG. 5 is a bar graph of the prevention of aggregation of Carboxypeptidase A by monoclonal antibody $CP_{10}$; aggregation of CPA, in the presence (bars with right slanting diagonal lines) and in the absence (single cross-hatch bars) of antibodies, was followed by determination of amount of mAb

bound to coated CPA in a competitive ELISA; the absorbance at 495 nm obtained in the absence of added soluble CPA was set at 100% for bound antibody; the soluble CPA, before heat exposure, competes with the coated CPA for antibody binding, leading to decrease in amount of antibody bound (60%) ([bars] bar with left slanting diagonal lines);

Please rewrite the paragraph at column 4, lines 45-51, as follows:

FIGS. 7A and 7B are a pair of graphs (A and B) showing aggregation of β-amyloid (1-40) in the absence (diagonal lines bars) and in the presence (open bars) of monoclonal antibodies AMY-33 (A) and 6F/3D (B) followed by ELISA; (1) β-amyloid alone, (2) ]β-amyloid+50] β-amyloid + 50 MM heparan sulfate, (3) [β-amyloid+10-3M] β-amyloid + $10^{-3}$M $AlCl_3$; (4) [β-amyloid+$10^{-3}$M] β-amyloid + $10^{-3}$M $ZnCl_2$; and

Please rewrite the paragraph at column 6, lines 61-67, as follows:

The expression vector can be a virus. Further the virus can be an RNA virus such as a disabled retro virus or a [retrovital] retroviral shuttle vector. The expression vector can also be vaccinia virus or an adenovirus. The expression vector can also be a plasmid. In a preferred embodiment wherein β-amyloid [in] is the targeted molecule the expression

C9 vector is selected that is known to target the central nervous system.

Please rewrite the paragraph at column 7, lines 13-22, as follows:

A specific example of DNA [vital] viral vector for introducing and expressing recombinant sequences is the adenovirus derived vector Adenop53TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative selection and an expression cassette for desired recombinant sequences. This vector can be used to infect cells that have an adenovirus receptor. This vector as well as others that exhibit similar desired functions can be used to treat a mixed population of cells and can include, for example, an in vitro or ex vivo culture of cells, a tissue or a human subject.

C10

Please rewrite the paragraph at column 7, lines 23-36, as follows:

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus such as antibiotic sensitivity. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the

C11

addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the [vital] viral vector or recombinant sequence, cellular transformation will not occur. Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

Please rewrite the paragraph at column 7, lines 37-50, as follows:

In addition, recombinant [vital] viral vectors are useful for in vivo expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original [vital] viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

Please rewrite the paragraph at column 7, lines 51-65, as follows:

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of vital vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. The vector to be used in the methods of the invention will depend on desired cell type to be targeted and will be known to those skilled in the art. For example, if breast cancer is to be treated then a vector specific for such epithelial cells would be used. Likewise, if diseases or pathological conditions of the hematopoietic system are to be treated, then a [vital] viral vector that is specific for blood cells and their precursors, preferably for the specific type of hematopoietic cell, would be used.

C13

Please rewrite the paragraph from column 7, line 66 to column 8, line 18, as follows:

Retroviral vectors can be constructed to function either as infectious particles or to undergo only a single initial round of infection. In the former case, the genome of the virus is modified so that it maintains all the necessary

C14

genes, regulatory sequences and packaging signals to synthesize new viral proteins and RNA. Once these molecules are synthesized, the host cell packages the RNA into new [vital] viral particles which are capable of undergoing further rounds of infection. The vector's genome is also engineered to encode and express the desired recombinant gene. In the case of non-infectious viral vectors, the vector genome is usually mutated to destroy the viral packaging signal that is required to encapsulate the RNA into viral particles. Without such a signal, any particles that are formed will not contain a genome and therefore cannot proceed through subsequent rounds of infection. The specific type of vector will depend upon the intended application. The actual vectors are also known and readily available within the art or can be constructed by one skilled in the art using well-known methodology.

Please rewrite the paragraph at column 8, lines 19-39, as follows:

If [vital] viral vectors are used, for example, the procedure can take advantage of their target specificity and consequently, do not have to be administered locally at the diseased site. However, local administration can provide a quicker and more effective treatment, administration can also be performed by, for example, intravenous or subcutaneous

injection into the subject. Injection of the viral vectors into a spinal fluid can also be used as a mode of administration, especially in the case of neuro-degenerative diseases. Following injection, the viral vectors will circulate until they recognize host cells with the appropriate target specificity for infection. Alternatively, the method as set forth by Tuomanen et al. (1993) can be used.

Please rewrite the paragraph at column 11, lines 13-18, as follows:

The enzymatic activities of CPA and its immunocomplexes were determined spectrophotometrically at 254 nm using either 1 mM hippuryl-L-phenylalanine as peptidase substrate or hippuryl-DL-β-phenyllactic acid as esterase substrate in 0.5M NaCl/0.05M [Tris-Hl] Tris-HCl, pH 7.5, (Solomon et al., 1989).

Please rewrite the paragraph at column 11, lines 20-23, as follows:

Amyloid peptides, Aβ 1-40 (Cat. No. A-5813) and Aβ 1-28 (Cat. No. A-1084) corresponding to amino acids 1-40 and 1-28 of Aβ respectively, were purchased from Sigma Chemical Co., St. Louis, Mo., USA[)].

Please rewrite the paragraph at column 11, lines 24-26, as follows:

Amyloid solutions were prepared by dissolving the peptides in water at concentration of 10 mg/ml. The stock solution was stored in [aliquotes] aliquots at -20° C.

C18

Please rewrite the paragraph at column 11, lines 28-31, as follows:

Heparan sulfate (Cat. No. H 5393) was purchased from Sigma Chemical Co., St. Louis, Mo., USA[)]. Stock solutions of metal chlorides were made up from dry salts at concentration of 1 mM in TRIS pH 7.4.

C19

Please rewrite the paragraph at column 12, lines 29-37, as follows:

The antigen-coating solutions (100 µl) containing native CPA (10-25 µl ml) in PBS, pH 7.4, were incubated overnight at 4° C. in a polystyrene ELISA plate (Costar, Cambridge, Mass.). Diluted ascites fluid (0.1 ml) containing the desired mAb (1:2000 to 1:18,000 v/v in PBS) was added and incubated at 37° C. for 1 hour. The amount of bound mAb was determined with β-galactosidase-linked $F(ab)_2$ fragments of sheep anti-mouse IgG (Amersham International, UK).

C20

Please rewrite the paragraph at column 12, lines 42-61, as follows:

CPA (10 µl/ml of PBS) was adsorbed onto ELISA plates overnight at 4° C., the remaining active groups on the plate being blocked with non-fat milk. To the soluble CPA (200 ng

C21

in 10 µl PBS), incubated for one hour at 50° C., the mAb $CP_{10}$ (molar ratio 1:1 Ab/CPA) was added and allowed to interact with the remaining soluble CPA for one hour at 37° C. In parallel, the mAb was added to the CPA solutions before exposure at 50° C. for one hour. After incubation, the CPA preparations were removed by centrifugation at 15,000 [rmp] rpm for 15 minutes and applied on the ELISA plates coated with CPA. The antibody which did not bind to soluble CPA in the reaction mixture will bind to the coated CPA; the amount of antibody bound to the coated antigen will be conversely proportional to the extent of CPA aggregation and determined using α-mouse antibodies labeled with horseradish peroxidase (HRP). The color developed by HRP ([0]O-phenylenediamine (OPD) as substrate) was measured at $OD_{495}$ using an ELISA plate reader. The amount of antibody bound on the coated CPA in the absence of soluble CPA was considered as 100%.

Please rewrite the paragraph at column 13, lines 20-40, as follows:

The ELISA plates were coated with rabbit polyclonal antibodies [(Boeringer-Mannheim)] (Boehringer-Mannheim) raised against synthetic [α-amyloid] β-amyloid (1-40) (Sigma) (100 ng/well) via covalent attachment to epoxy-coated ELISA plates by incubation at 4° C. for 16 hours. The residual epoxy groups were blocked by non-fat milk. The reaction mixtures

containing aqueous solution of [α-amyloid] β-amyloid (100 ng/ml), heparan sulfate (50 mM) and/or chloride metal solutions ($10^{-3}$ M at pH 6.5), were incubated at 37° C. for three hours. The aggregated β-amyloid preparations were removed by centrifugation at 15,000 g for 15 minutes. The residual soluble β-amyloid was incubated for another one hour at 37° C. with mAbs AMY 33 and/or 6F3D at equal molar ratio antibody/antigen. In another set of experiments, the mAbs were added to the reaction mixtures before incubation at 37° C. and then incubated together for 3 hours at 37° C. After the incubation period, the immunocomplexed amyloid preparations were added to the ELISA plates, previously coated with polyclonal anti-amyloid antibodies. The amount of mAb bound will be proportional to the amount of soluble amyloid which remained after exposure to aggregation conditions.

Please rewrite the paragraph at column 13, lines 41-50, as follows:

The amount of bound antibody was determined using [a-mouse] α-mouse second antibodies labeled with horseradish peroxidase (HRP). The enzyme activity of HRP is directly proportional with the amount of residual amyloid bound to rabbit polyclonal antibodies. The enzyme activity of HRP was measured using O-phenylenediamine (OPD) as substrate. The color developed was measured at $A_{495}$ using an ELISA reader.